REVIEW ARTICLE

Parasitic helminths: a pharmacopeia of anti-inflammatory molecules

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SUMMARY

Infection with parasitic helminths takes a heavy toll on the health and well-being of humans and their domestic livestock, concomitantly resulting in major economic losses. Analyses have consistently revealed bioactive molecules in extracts of helminths or in their excretory/secretory products that modulate the immune response of the host. It is our view that parasitic helminths are an untapped source of immunomodulatory substances that, in pure form, could become new drugs (or models for drug design) to treat disease. Here, we illustrate the range of immunomodulatory molecules in selected parasitic trematodes, cestodes and nematodes, their impact on the immune cells in the host and how the host may recognize these molecules. There are many examples of the partial characterization of helminth-derived immunomodulatory molecules, but these have not yet translated into new drugs, reflecting the difficulty of isolating and fully characterizing proteins, glycoproteins and lipid-based molecules from small amounts of parasite material. However, this should not deter the investigator, since analytical techniques are now being used to accrue considerable structural information on parasite-derived molecules, even when only minute quantities of tissue are available. With the introduction of methodologies to purify and structurally-characterize molecules from small amounts of tissue and the application of high throughput immunological assays, one would predict that an assessment of parasitic helminths will yield a variety of novel drug candidates in the coming years.

Key words: helminths, immunomodulators, anti-inflammatory drugs, drug design.

INTRODUCTION

Infections with parasitic helminths continue to cause considerable morbidity and mortality in humans and their domestic livestock, translating into substantial socioeconomic losses. For instance, morbidity associated with *Schistosoma mansoni* or *Echinococcus granulosus* infections is debilitating and sometimes fatal (Carod-Artal, 2008; King and Dangerfield-Cha, 2008), and infections with hookworms retards the growth of children and can impair their cognitive functions (Oberhelman *et al*. 1998). The magnitude of the problems is illustrated by estimates from the the World Health Organization (WHO, 1999) that, even in the midst of successful anthelmintic programs, 30% of the world's population harbours at least 1 species of helminth parasite; a staggering 2 billion people are affected! However, numerous studies have shown that modulation of the immune response of the host by parasitic helminths can have a concomitant health benefit (reviewed by McKay, 2006). In addition to infections with live organisms, helminth-derived molecules are potent immunomodulators and can serve as templates for the design of novel anti-inflammatory drugs, and may also be candidates for vaccine development (Harnett and Harnett, 2008; Perrigoue *et al*. 2008). The former aspect of the host-parasite interaction is the topic of this review.

HELMINTH THERAPY: THE GOOD, THE BAD AND THE WRIGGLY

Parasitic helminths have been humans' long-time companion and have evolved as 'master regulators' of host immune responses (Maizels *et al*. 2004). Consequently, knowledge of how the parasite modifies host immunity can be used to develop therapies for other diseases. In considering 'helminth therapy', one can either use viable infections or immunomodulatory molecules from the parasite; both strategies have been used to treat diseases in animal models. Infection with a variety of species of

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parasitic helminths has been shown to reduce the severity of airway, cerebral and intestinal inflammation in murine models of human disease (McKay, 2006), and a number of investigators are pursuing the mechanism(s) underlying this health benefit (Weinstock *et al*. 2005). Moreover, Weinstock and colleagues have shown that the ingestion of viable eggs of *Trichuris suis* by patients with inflammatory bowel disease (Crohn's disease and ulcerative colitis) led to a reduction in disease activity, and adverse side-effects were not apparent (Summers *et al*. 2003, 2005). However, there can be a number of concerns regarding the use of an infection as a therapy. First, a parasite that has limited impact on the host must be used, which prohibits the use of particular species, such as *S. mansoni*, despite its potent stimulation of host immunity. Second, there are patient groups for which this approach would not be advocated, typically children, the elderly, immunocompromised individuals and pregnant women (Farah *et al*. 2007). Third, there are examples of helminth infections worsening disease evoked by bacteria or protozoa (Chen *et al*. 2005; Marshall *et al*. 1999) and, in at least one case, chemically-induced colitis (Hunter *et al*. 2007). Finally, when introducing a species to a novel environment, there is the potential for maladaptive responses and unpredicted pathological effects/changes in remote tissues. Moreover, the possibility exists for parasite establishment in, rather than expulsion from the host (Kradin *et al*. 2006). Despite these issues, the data on 'helminth therapy' are encouraging, and animal models can be used to identify novel anti-inflammatory mechanisms for translation into medical treatments, negating the need for actual infection.

The use of helminth-derived molecules as drugs overcomes the concerns with the use of live parasites. However, the issues here are often technical and revolve around a single basic question: how can pure molecules be isolated from miniscule amounts of parasite material to allow testing in biological systems and to serve as blueprints for drug development?

IMMUNE RESPONSES TO HELMINTH PARASITES: THE BASICS

A number of authorative reviews of the mammalian immune system and the responses to infection with helminths are available (Hayes *et al*. 2004; Maizels *et al*. 2004; Antony *et al*. 2007; Khan, 2008; Perrigoue *et al*. 2008). As metazoans, helminths cannot be ingested by macrophages and other phagocytes, so that a variety of effector mechanisms have evolved to combat helminths. These involve the mobilization and activation of T and B lymphocytes, mast cells, eosinophils, natural killer (NK) cells, and, if the infection is at a mucosal surface, mucus-producing goblet cells. Briefly, while there is likely to be an innate immune response to helminth-derived products by macrophages and natural killer cells, the current view is that the predominant anti-worm response is the remit of an adaptive immune response. Thus, following antigen processing and presentation by professional antigen presenting cells (e.g., dendritic cells (DCΦs)), humans and mice display a stereotypic T cell response which is characterized by the development of $CD4^+$ T helper (TH)-2 cells that produce interleukin (IL)-4, -5, -13 and others. These chemical signals mobilize other cells; for example, they direct isotype switching, so that high affinity immunoglobulin (Ig) G, E and A can be produced. These antibodies then bind to receptors on effector cells, allowing for their activation by worm antigen and the release of molecules (e.g., mast-derived histamine and trypases, eosinophil-derived cationic protein) that either disrupt the surface of the helminth and/or alter the environment, making it less hospitable to the helminth. In addition, IgG can bind directly to the surface of the helminth (opsonization), interfering with worm behaviour and targeting the worm for attack by complement and polymorphonuclear cells. Thus, the host response to infection with parasitic helminths is multi-cellular and is orchestrated by Th2 cells/cytokines. Recent studies are elucidating a role for additional cytokines such as IL-21 and IL-33 (Humphreys *et al*. 2008) and regulator cells, including the $CD4^+$/$Foxp3^+$ natural regulatory T cells and, alternatively activated macrophages (AAM) (Baumgart *et al*. 2006; Taylor *et al*. 2006; Persaud *et al*. 2007). While all immune cells can be considered as immunomodulatory (i.e., they affect other immune and stromal cells in their vicinity), regulatory T cells and AAMs (of which there are multiple types for each; Belkaid and Oldenhove, 2008; Reyes and Terrazas, 2007) have emerged as prototypic immunomodulators whose primary function is to switch-off or dampen immune reactions, or engage reactions that promote tissue recovery and restitution. Much research remains to be done to precisely define the biology of regulatory T cells and AAMs; however, the mobilization of both classes of cells following an infection with helminths would generate an immunoregulatory/immunosuppressive environment in which a spectrum of immune events would be inhibited. Thus, by extrapolation, the ability of products from the helminth to elicit regulatory T cell or AAM activity could contribute to worm infectivity and survival.

HELMINTH-DERIVED IMMUNOMODULATORS

An analysis of the biology of a parasite should include studying the mechanisms of the interaction of the parasite with the host immune system, so that potential parasite-derived immunomodulators (used to develop drugs to treat immuno-pathological

changes/disease) can be identified. During the last 40 years, a staggering number of helminth-derived immunomodulators have been partially characterized, both in terms of structure and bioactivity (we define an 'immunomodulator' as any molecule that regulates host immunity, but not antigens recognized by the host that are used to generate immune responses aimed at the destruction or elimination of the parasite from the host). Here, we focus on selected examples from the 3 main groups of parasitic helminths and draw the reader's attention to the fact that a literature search will reveal many other examples, all worthy of discussion, and excluded only on the basis of space restrictions (the examples given below are supplemented by additional material in Tables 1–3).

Trematodes

Schistosoma spp. Schistosomes, because of their impact on human health (King and Dangerfield-Cha, 2008), have been the focus of intense research efforts, and a variety of glycoconjugates have been extracted from the eggs and adult tegument (Bennett, 1963). Soluble egg antigen (SEA) from *S. mansoni* has been implicated in the host-response to infection, and has been shown consistently to have immunomodulatory properties. For example, SEA appears to be responsible for the reduction in $CD4^+$ lymphocytes 6 weeks following infection, an event that was attributed to enhanced Fas-FasL-induced apoptosis involving $CD19^+$ B cells (Lundy *et al.* 2001). Excretory/secretory (ES) products from schistosomula contain a 23 kDa molecule that results in T cell depletion *in vivo via* Fas-FasL killing. Exposure to SEA results in reduced levels of nitric oxide (NO) and T helper cell-type 1 (TH1) chemokines (CCL3) and cytokines (tumour necrosis factor-α (TNFα), IL-12) and an increased expression of markers indicative of AAMs. These events are all compatible with the mobilization of TH2-type cytokines (e.g. IL-4, IL-13) which is stereotypic of the mammalian immune response to infection by helminth parasites. The induction of AAMs is of particular note, as these cells exert anti-inflammatory effects and are active in tissue restitution after injury (Gordon and Taylor, 2005). Thus, the ability to use a consitutent of SEA to induce this cellular response pharmacologically could be of direct health benefit.

Mice treated with SEA display endothelial cell proliferation and capillary formation which appear to be driven by a heat- and protease-insensitive molecule, indicative of a glycan (Kanse *et al.* 2005). These findings complement the work of van de Vijver *et al.* (2006) that detected LDN (GalNAcβ1, 4GlcNAc) and LN (Galβ1,4GlcNAc) modified glycans in SEA-stimulated granulomata. SEA has been shown to induce vascular endothelial growth factor (VEGF) secretion (Loeffler *et al.* 2002) and fibroblast-stimulating factor (FSF-1) (Greenwel *et al.* 1993), which could contribute to the formation of the fibrous capsule around the granulomata, although it is unclear whether the stimulation of the production of either growth factor was due to a glycan component of the SEA. Nevertheless, one could speculate that SEA evoked growth factor release from host cells to stimulate angiogenesis could be used to enhance the recovery from tissue damage, although the potential to elicit metastatic events should not be overlooked.

In addition, while the exposure to SEA decreased the expression of Toll-like receptors (TLR) on macrophages, the responses of these cells to TLR agonists (e.g., lipopolysaccharide (LPS)) were not appreciably retarded (Joshi *et al.* 2008). These studies are intriguing, as they demonstrate a cross-talk between infection with parasitic helminths and responsiveness to bacteria and may illuminate a way in which one could exploit one type of infection to treat another. In addition, the complexity of the host response to infection, which is linked temporally or spatially and/or parasite-specific, is aptly illustrated by these studies.

The identification of a >100 kDa heat-insensitive, carbohydrate-positive fraction from *S. mansoni* (see Hayunga *et al.* 1979) was followed by studies characterizing the glycoproteins of schistosomes and notably led to the elucidation of lacto-N-fucopentose (LNFPIII) (carbohydrate containing the mammalian $Lewis^X$ trisaccharide [Galβ1,4[Fucα1,3] GlcNAc]) and lacto-N-neotetrose (LNT) (Ko *et al.* 1990). Analysis of LNT revealed that its injection into mice caused an expansion of a $Gr1^+$ cell population which suppressed $CD4^+$ T cell proliferation and cytokine production *via* an apoptosis-independent but cell contact-dependent mechanism (Terrazas *et al.* 2001). The authors postulated that this treatment 'imprinted' the $Gr1^+$ cells with a TH2-type phenotype.

Since the $Lewis^X$ antigen is present in the lymphocyte function-associated molecule-1 (LFA-1), the role of LNFPIII in lymphocyte proliferation was assessed and shown to stimulate IL-10 and prostaglandin E_2 (PGE_2) production from B-cells of infected mice without hampering antigen presentation (Velupillai and Harn, 1994). Also, the co-culture of LNFPIII-treated macrophages and NK cells enhanced NK cell activation as gauged by the expression of CD69 and interferon-γ (IFNγ) production (Atochina and Harn, 2005). Lacto-N-fucopentose III also generates anti-inflammatory responses by signalling through Toll-like receptor 4 (TLR4) to induce the phosphorylation and activation of the mitogen-activated protein kinase (MAPK), ERK (Thomas *et al.* 2003).

The presence of fucose in the $Lewis^X$ moiety of LNFPIII is essential for bioactivity (Okano *et al.*

Table 1. Selected examples of immunomodulatory molecules from trematodes

Species	Worm product	Bioactivity	Reference
Schistosoma japonicum	900 kDa ECF-SjE from homogenized eggs; pronase and heat insensitive glycoprotein; destroyed by periodate oxidation	*In vitro* eosinophil chemotaxis	Owhashi and Ishii (1982)
	440 kDa JAE-H and <440 kDa JAE-L glycoproteins from adult ES products	Eosinophil chemotactic factors; JAE-L also induces neutrophil chemotaxis	Horii *et al*. (1984)
Schistosoma mansoni	Analogues of adrenocorticotrophin (ACTH) and α-melanotrophin (α-MSH)	ACTH converts to α-MSH by polymorphonuclear cells via neutral endopeptidase; αMSH inhibits leukocyte adherence and immunosuppressive	Duvaux-Miret *et al*. (1992)
	Various surface glycans	Fucose and Galactose linked to bovine serum albumin reduced ERK and PKC phosphorylation and phagocytosis in *Lymnaea stagnalis* haemocytes	Plows *et al*. (2005)
	25 kDa glutathione S-transferase in ES products	Detoxification of peroxides involved in oxidative stress	Guillou *et al*. (2007)
Fasciola hepatica	ES products contain metal ion dependent glycosidases (β-galactosidase, β-N-acetylhexosaminidase and β-glucosidase)	May degrade host mucins rich in galactose, N-acetylglucosamine and N-acetylgalactosamine	Irwin *et al*. (2004)
	ES products	Prevent superoxide production by PMA-activated sheep and human neutrophils *in vitro* (a heat resistant ES component from the related species, *F. gigantica*, does the same)	Jefferies *et al*. (1997) El-Ghaysh *et al*. (1999)
Diplostomum pseudopathaceum	22–24 kDa lectin with homology to β1,3 glucan binding protein localizes to the cercarial penetration glands	Agglutinates murine red blood cells and may facilitate tissue recognition and penetration	Mikes and Horak (2001)
Paragonimus westermani	27 kDa cysteine protease in ES products	Induces superoxide production and human eosinophil degranulation	Chung *et al*. (2008)
Opisthorchis viverrini	24 kDa thioredoxin peroxidase (TPx) isolated by genomic probing; also exists in ES products	Protects worm from reactive oxygen metabolites and may have other roles similar to *F. hepatica* TPx	Suttiprapa *et al*. (2008)

1999), since lacto-N-neotetrose lacking Lewis^X had no effect on DCΦ differentiation to an IL-4 producing phenotype in response to LPS. Importantly, the induction of accessory molecules is required to increase the strength of an immune response; DC-SIGN has strong affinity for intercellular adhesion molecule-3 (ICAM3) via the Lewis^X structure (Bogoevska *et al*. 2007), suggesting a possible mechanism used by LNFPIII to bind to DCΦ and interfere with cell-to-cell communication with other immune cells (Appelmelk *et al*. 2003). The multivalency of LNFPIII is important for its bioactivity, since monovalent LNFPIII was shown to be incapable of inhibiting antigen-induced peripheral blood mononuclear cell (PBMC) activation (Velupillai *et al*. 2000). At the signalling level, LNFPIII increases nuclear factor (NF)-κB activity in DCΦ to a level similar to LPS; however, a second phase (prolonged NF-κB activation associated with IκBβ degradation) was not observed. Use of the nuclear import inhibitor, SN50, and NFκB-deficient mice revealed that LNFPIII effects on DCΦ were indeed NFκB-dependent (Thomas *et al*. 2005). Collectively, these studies suggest that LNFPIII (or possibly fragments thereof) could be used to extrinsically manipulate DCΦ function as a way of limiting immunopathological reactions/changes or controlling immune cell responsiveness.

The stimulation of IL-10 and IL-12p40 from murine peritoneal macrophages by cercarial ES products required the presence of TLR4, whereas the production of IL-6 was TLR4 independent (Jenkins *et al*. 2005). Furthermore, IL-6 release was reduced following protease treatment of the ES products suggesting a proteinaceous ligand, and sodium periodate ablated activity of the cercarial extract, indicating the presence of glycans. It is plausible that cercarial products contain proteins/glycoprotiens that can act *via* TLR4, highlighting the promiscuity of TLR4 as more than an LPS receptor. Other TLRs have been implicated as receptors for schistosome ligands. Lyso-phosphatidlyserine from *S. mansoni* induces DCΦ maturation and decreases IL-12p70 production *via* TLR2. The activity is abrogated by TLR2-blocking antibodies (van der Kleij *et al*. 2002), whereas host phosphatidylserine neither activates TLR2 nor induces DCΦ maturation. Additionally, phosphoserine alone is not effective in this system, indicating that the acyl and phosphoserine groups of *S. mansoni* lysophosphatidylserine are required for the effect. Inhibition of lysophosphatidylserine acyltransferase (LPC-AT) did not affect TLR4 expression or the binding of LPS, but it did prevent TLR4 translocation to lipid rafts and thus blocked the formation of the TLR4 complex with a resultant decrease in TNFα and IL-6 production (Jackson *et al*. 2008). It is possible that *S. mansoni* lysophosphatidylserine also signals through TLR4 in a similar manner. Studies such as these illustrate an emergent theme, in which reponses to helminth-derived molecules occur *via* receptors that have been defined on the basis of their ability to recognize conserved microbe-derived products; however, the data must be interpreted with caution, since the possibility of bacterial contamination of the helminth extracts is omnipresent.

Fasciola hepatica. ES products from adult *F. hepatica* (*Fh*ES) contain a glycan that is cytotoxic to eosinophils and acts *via* tyrosine kinase and caspase-dependent mechanisms (Serradell *et al*. 2007). Induction of AAMs often accompanies an infection with helminths, and it has been reported that *F. hepatica* thioredoxin peroxidase elicits AAMs production (Donnelly *et al*. 2005). Numerous studies have shown that AAMs are induced by IL-4/IL-13 (Stein *et al*. 1992); however, in the case of *F. hepatica* thioredoxin peroxidase, this conversion appeared to be IL-4-independent (Donnelly *et al*. 2005). The peroxidase also increased transforming growth factor-β (TGFβ) and PGE_2 while decreasing IL-10, IL-12 and IL-18 from stimulated macrophages – such events *in vivo* would skew the immune reponse in favour of immunoregulation/immunosuppression and away from TH1-dominated pro-inflammatory reactions. Complementing these findings, the *in vitro* treatment of bovine macrophages with *Fh*ES products inhibited IFNγ and NO production in response to bacterial products and skewed the macrophage population towards an AAM phenotype; the latter event was lost by heat treatment of the ES products (Flynn and Mulcahy, 2008). This emphasizes that we must constantly be vigilant of the possibility that parasite-derived molecules, as well as those produced by the host (e.g., cytokines), can modify the host's immunity. Finally, cathepsin L appears to be a dominant molecule in *Fh*ES and may aid *F. hepatica* by degrading host tissue to assist parasite entry/migration, and interfering with the host's immune response by, for example, the degradation of IgG (Smith *et al*. 1993; O'Neill *et al*. 2001).

Cestodes

Pseudophyllidean and cyclophyllidean tapeworms usually elicit limited damage in the alimentary tract of their definitive host. However, they are recognized by their host (i.e., anti-worm antibodies are generated) so, the ability to suppress or negate host immunity would remain a priority for the helminth (see Table 2).

Taenia crassiceps. T cells from *T. crassiceps*-infected mice are hypo-proliferative and, in the presence of excretory/secretory (*Tc*ES) products, show diminished responses to mitogen stimulation *in vitro* (Spolski *et al*. 2000, Scuitto *et al*. 1995). Administration of *Tc*ES products to mice caused expansion of F4/80$^+$ Gr1$^+$ cells, and these cells

Table 2. Selected examples of immunomodulatory molecules from cestodes

Cestode	Worm product	Bioactivity	Reference
Echinococcus multilocularis	ES product	Modifies macrophage accessory function leading to inhibition of lymphocyte proliferation *in vitro*	Rakha *et al*. (1991)
	Metacestode glycosphingolipids	Inhibits PBMC proliferation; decreases IL-2/IL-2 receptor expression	Persat *et al*. (1996)
	14-3-3 proteins in ES products	Inhibits LPS-induced nitric oxide production by rat macrophages	Andrade *et al*. (2004)
Echinococcus granulosus	E4 Galα1,4Gal glycan from protoscolesces extract	Inhibits mitogenic-induced proliferation; increases IL-10	Dematteis *et al*. (2001)
	Antigen B protease inhibitor in hydatid cyst fluid	Inhibits neutrophil recruitment *in vitro*; increased production of IL-4 and IL-13 by human PBMCs; impairs human DCΦ differentiation but polarizes maturation of immature DCΦ to TH2	Shepherd *et al*. (1991) Rigano *et al*. (2001, 2007)
Taenia multiceps	Glucose lectin in cyst fluid	May have similar function as ES product of *E. multilocularis*	Judson *et al*. (1987)
Taenia taeniaeformis	19·5 kDa proteinase inhibitor from somatic extract	Inhibits splenocyte proliferation in response to mitogenic and antigenic stimuli	Leid *et al*. (1984, 1986) Suquet *et al*. (1984)
	ES products	Sequesters complement; decreases mitogen-induced splenocyte proliferation IL-2 production	Rikishia *et al*. (1985) Burger *et al*. (1986)
Taenia crassiceps	Interferon-γ analogue (p66) in ES products	Enhances mitogen-induced splenocyte proliferation and IFNγ and IL-10 production; increases nitric oxide production by macrophages increases	Spolski *et al*. (2002)
Taenia solium	RNA peptide in metacestode extracts	Inhibits response to *Salmonella* antigens as well as mitogen-induced proliferation and inflammatory responses to metacestode antigens, correlating with decreased IL-2, IFNγ and IL-4; reduced TNFα production by macrophages following LPS stimulation	Arechavaleta *et al*. (1998)

were capable of blocking T cell activation *in vivo*. Glycans in the *Tc*ES were essential for this response, since the treatment with sodium periodate significantly reduced F4/80$^+$ Gr1$^+$ cells in the peritoneal cavity of infected mice and the production of cytokines and AAM (Gomez-Garcia *et al*. 2005). Extracts from the adult worm also directed a strong TH2 polarization (IL-4 production) and enhanced antibody production (IgG_1 and IgE) in mice co-treated with ovalbumin (Gomez-Garcia *et al*. 2006). Concomitant with these events was an increased expression of markers indicative of AAMs, a predicted decrease in IFNγ and iNOS expression and increased synthesis of IL-10 and TGFβ. Like the ES products, the glycan component was critical for these effects of a crude antigen preparation from the adult worm, because all were abolished or significantly inhibited by sodium periodate treatment of the extract.

Extracts from the *T. crassiceps* metacestodes are highly mannosylated and contain both non-fucosylated and fucosylated structures, including the Lewis^X antigen (Dissanayake *et al*. 2004). In contrast to the adult worm antigen, the effect of which was unaltered by the knock-out of TLR4 (Gomez-Garcia *et al*. 2005), the ability of the glycans in the metacestode extract to elicit IL-6 production from murine peritoneal macrophages was reduced in cells lacking TLR2 and TLR4 (Dissanayake *et al*. 2004). The same researchers recently found that the terminal beta-(1-4)-galactose residue in the Lewis^X analogue can evoke IFNγ production by murine splenocytes and activate the NF-κB pathway. The IFNγ production was reduced by inclusion of anti-TLR2, -4 and -6 antibodies in the assay (Dissanayake and Shahin, 2007), again reiteriating the notion that helminth-derived products can exert their effects *via* TLRs.

Spirometra erinaceieuropaei. Excretory/secretoy (*Se*ES) products contain immunoglobulinases and a heat-sensitive factor that inhibits LPS- and lipoteichoic acid (LTA)-induced TNFα and IL-1β production from murine macrophages (Diragahayu *et al*. 2004). The inhibitory effect of the *Se*ES products was not due to the stimulation of PGE_2, IL-10 or TGFβ in the target cells (Fukushima *et al*. 1993). The inhibition of macrophage production of TNFα, IL-β and NO was also shown in a study by Kina *et al*. (2005), in which a 90 kDa trypsin-sensitive glycoprotein was identified as the active component in the *Se*ES products. Similarly, a 94 kDa component of *Se*ES products could block LPS-induced MAPK (i.e., ERK1, ERK2 and p38MAPK) phosphorylation and the accompanying synthesis of TNFα (Dirgahayu *et al*. 2002).

Hymenolepis diminuta. Extracts from adult *H. diminuta* reduced ConA-induced proliferation, IL-2 and IL-4 synthesis by murine spleen cells and human PBMC cells in a concentration-dependent manner (Wang and McKay, 2005). These events were mirrored, to a large extent, by use of ES products from the adult worm. A crude phosphate-buffered saline (PBS)-solube extract of the adult worm stimulated IL-10 release from murine splenocytes and also contained a component with some structural similarity to the p40 chain of IL-12, as detected by enzyme-linked immunosorbent assay (ELISA) and immunoblotting. The bioactivity in both the worm extract and the ES products was within a >50 kDa fraction and was partially sensitive to boiling (Wang and McKay, 2005).

Nematodes

Parasitic nematodes have been the focus of extensive research efforts because of their major socio-economic impacts globally (Hall *et al*. 2008). Therefore, a number of immunomodulatory molecules have been identified from this phylum (Table 3). We focus this discussion by highlighting known molecules from *Nippostrongylus brasiliensis*, *Trichinella spiralis*, *Necator americanus*, *Trichuris suis*, *Ascaris suum* and *Acanthocheilonema viteae*.

Nippostrongylus brasiliensis. ES products from this parasite direct a range of immunomodulatory reactions. As examples, (i) they reduce LPS-induced IL-12p40 production and expression of a panel of cytokine/chemokine genes in murine bone-marrow-derived DCΦ, (ii) an undefined protein component induces IL-4, IL-10 and IgE production, with the IL-4 response requiring the presence of major histocompatibility class II (MHC II) antigens and so presumably MHC II-T cell restricted events, and (iii) suppress TH1-events via an IL-10-independent mechanism (Holland *et al*. 2000, 2005; Balic *et al*. 2004). The characterization of the ES products revealed the presence of a serine acetylhydrolase capable of reducing intestinal anaphylactic responses by inactivating platelet activating factor and blocking neutrophil and eosinophil aggregation (Blackburn and Selkirk, 1992). The value of inhibiting eosinophil activity to the helminth is intuitive, and the use of this acetylhydrolase would be beneficial in limiting the pro-inflammatory effects of granulated phagocytes in other diseases. Furthermore, *Nb*ES blocked goblet cell hyperplasia, eosinophilia, neutrophilia and airway hyper-responsiveness in murine models of asthma (Keir *et al*. 2004; Trujillo-Vargas *et al*. 2007). The active component/s was/were heat and protease insensitive, suggesting a glycan-type molecule. When *Nb*ES was administered intraperitoneally to ovalbumin-sensitized mice, they developed less pathology on ovalbumin re-challenge than untreated controls; in contrast, intranasal *Nb*ES induced significant lung inflammation (Marsland

Table 3. Selected examples of immunomodulatory molecules from nematodes

Nematode	Worm product	Bioactivity	Reference
Ascaris suum	Pseudocoelomic fluid protein(s)	Induces eosinophil and neutrophil chemotaxis; reduces bronchoalveolar lavage fluid cell infiltrate, IL-5 and IL-13 as well as expression of CD40 and CD86 and is therapeutic in murine asthma models	Tanaka *et al*. (1979) McConchie *et al*. (2006)
	Somatic extract from homogenized adults	Inhibits zymosan-induced murine arthritis; reduces hypersensitivity to ovalbumin independently of IL-10, and reduces cell proliferation, IL-2, IFNγ, eosinophil peroxidase, IL-4, IL-5 and eotaxin levels;	Rocha *et al*. (2008) Souza *et al*. (2002) Lima *et al*. (2002)
	200 kDa PAS-1 from adult somatic extract	Inhibits airway allergy induced by the worm allergen via inhibition macrophage cytokine release	Itami *et al*. (2005)
	Phosphorylcholine-conjugated glycosphingolipids from adults	Increases TNFα, IL-1β and IL-6 from human PBMCs; decreases B cell proliferation; is pro-apoptotic; decreases TH1 cytokine production from LPS + IFNγ treated macrophages	Deehan *et al*. (2002) Kean *et al*. (2006) Katz *et al*. (2001)
Anisakis simplex	Heat sensitive larval homogenate	Eosinophil chemotaxis	Tanaka and Torisu (1978)
Brugia malay	47 kDa serine protease inhibitor in ES products	May inhibit neutrophil cathepsin G and elastase (disputed by Stanley and Stein (2003))	Zang *et al*. (1999)
	62 kDa asparaginyl-tRNA synthetase in ES products	Induces ERK1/2 MAPK phosphorylation via a G-protein coupled receptor; chemotactic for immature DCΦ, lymphocytes, eosinophils, neutrophils and cells positive for chemokine receptors CXCR1 and CXCR2,	Kron *et al*. (1995) Ramirez *et al*. (2006)
	Galectin-1 like molecule in ES products	Binds galactose containing glycoconjugates and may protect helminth from eosinophil and neutrophil mediated damage	Hewitson *et al*. (2008)
	12 kDa macrophage migration inhibitory factor (MIF) in ES products	Likely to have similar function as *T. spiralis* MIF	Pastrana *et al*. (1998)
Necator americanus	ES products contain calreticulin	Identical to *H. contortus* calreticulin; C1q complement is bound which protects the nematode from surface damage while promoting feeding by sequestering pro-coagulants. May also bind C-reactive protein.	Kasper *et al*. (2001)
Trichinella spiralis	Encysting stage produces a 12 kDa macrophage migration inhibitory factor (MIF)-like molecule	Potential to regulate host macrophage responses	Wu *et al*. (2003)
Toxocara canis	O-glycans in ES products	May act as antibody decoys or to alter viscosity of host mucins	Gems and Maizels (1996) Loukas *et al*. (2000*b*)
	Tumour associated antigen ((GalNAc-O-Ser/Thr) (Tn)) and GalNAc polypeptide N-acetylgalactosaminyltransferase (ppGaNTase)	The antigen is synthesized by the enzyme via abnormal elongation of O-glycans and interacts with macrophage C-type lectins and affects T cell activation	Clausen and Bennett (1996) Casaravilla *et al*. (2003)
	ES products	Impair eosinophil-mediated damage through unknown mechanism	Giacomin *et al*. (2008)
Teladorsagia circumcincta	Adult and L4 ES products contain thioredoxin peroxidase (TPx)	Likely to have similar function to *F. hepatica* TPx in affecting TH1/TH2 cytokine balance	Craig *et al*. (2006)

Dictyocaulus viviparous	Possesses immunogenic N-glycan conjugated to phosphorylcholine as well as platelet activating factor	The latter may induce anti-PAF antibodies which may protect against eosinophil-mediated damage	Kooyman *et al.* (2007)
Haemonchus contortus	66 kDa glycoprotein in ES products	Inhibits monocyte function	Rathore *et al.* (2006)
	55 kDa glycoprotein in ES products	Identical function as *A. caninum* 41 kDa product	Anbu and Joshi (2008)
	62 kDa calreticulin in ES products	Prevents erthyrocyte lysis and binds pro-coagulant Factor X, Ca^{2+} and p24 which enables blood feeding by the worm; p24 has homology with C-reactive protein which is enhanced during helminth infection and induces tegumental damage via complement	Taylor and Hoole (1997) Kasper *et al.* (2001) Suchitra and Joshi (2005) Suchitra *et al.* (2008)
Heligmosomoides polygyrus	ES products	Similar anti-asthma activity as *N. brasliensis* extracts except in this case the effects are mediated by IL-10 from $CD4^+$ $CD25^+$ $Foxp3^-$ cells	Segura *et al.* (2007)
Ancyostoma caninum	41 kDa glycoprotein in ES products	Inhibits neutrophil adhesion and blunts hydrogen peroxide production via CD11b/CD18 integrin	Moyle *et al.* (1994)

et al. 2005). Finally, and intriguingly, *N. brasiliensis* may be worthy of consideration in cancer therapy. When carcinogenic cells were injected with *N. brasiliensis* extracts, eosinophils were recruited and attacked the cells (i.e., as if they were a helminth), resulting in reduced size of the carcinoma (Frenoy, 2005).

Trichinella spiralis. Structural and biochemical analysis of *T. spiralis* ES products (*Ts*ES) from larvae or adult worms revealed the presence of a nucleoside 5′-diphosphate phosphohydrolase (apyrase, 17 kDa), a 5′nucleotidase that preferentially targets AMP to generate adenosine and an adenosine deaminase (Gounaris, 2002; Gounaris *et al.* 2004). The ability to regulate AMP metabolism has a number of potential immunomodulatory effects, since adenosine is known to inhibit platelet aggregation, monocytes/macrophage TH1 cytokine production, and neutrophil synthesis of reactive oxygen species (Liaudet *et al.* 2002; Antonioli *et al.* 2008), and is protective in a murine model of colitis (Mabley *et al.* 2003). Also, adenosine stimulates epithelial electrogenic Cl^- secretion, which creates a driving force for water to move into the gut (Kottgen *et al.* 2003) and could act to flush *Trichinella* larvae as well as intestinal bacteria and protozoan pathogens from the host.

Necator americanus. Nematode-derived proteases serve a number of vital roles in larval exsheathment, the digestion of host tissue (facilitating invasion and migration) and in immune evasion (Gamble *et al.* 1989, McKerrow *et al.* 1990). *Necator americanus* ES (*Na*ES) contains at least 7 cysteine proteases, 1 serine protease and various metalloproteases, some of which act as immunoglobulinases (Kumar and Pritchard, 1992). These enzymes exert a range of bioactivities, the majority of which are unknown. However, one can speculate that the cleavage of immunoglobulins (Igs) could result in reduced complement activation and that the activity of cells that bind Igs and are activated by antibody-cross-linkage by antigen (e.g., mast cells and eosinophils) would be reduced. Eosinophil recruitment *in vivo* could also be perturbed by, for example, the degradation of the chemokine, eotaxin-1 (Culley *et al.* 2000). Additionally, a *Necator americanus* protein was shown to evoke release of IFNγ from murine and human NK cells but not NK T cells (Hsieh *et al.* 2004).

Trichuris suis and Ascaris suum. *Trichuris suis* ES (*Trs*ES) products contain heat-stable and protease-insensitive components which reduce the growth of the bacteria *Campylobacter jejuni*, *Escherichia coli* and *Staphylococcus aureus* (see Abner *et al.* 2001). High performance liquid chromatography (HPLC) did not reveal the presence of antibiotics (i.e., penicillin, streptomycin and amphotericin) in the

*Trs*ES. The active component was <10 kDa in size. *A. suum* pseudocoelomic fluid (PCF) also contained a 6–14 kDa heat-stable, trypsin-sensitive molecule which could kill Gram-positive bacteria (e.g., *S. aureus*) (Wardlaw *et al.* 1994). The molecule was designated ASABF (*Ascaris suum* antibacterial factor) (Kato and Komatsu, 1996) and is similar to insect defensins which penetrate Gram-positive bacterial membranes. Genes encoding antibacterial cecropins had been characterized in *A. suum*, and chemically synthesized versions are active against numerous species of bacteria (Pillai *et al.* 2005). With increasing incidence of antibiotic-resistant bacteria, the putative usefulness of these nematode-derived molecules is apparent.

Acanthocheilonema viteae. Nematode-derived cystatins (cysteine protease inhibitors) have been repeatedly shown to regulate immune cell activity. Cystatins from *A. viteae* (as well as those of other filarioids, including *Onchocerca volvulus* and *Brugia pahangi*) inhibit T cell proliferation, interfere with antigen processing and presentation, and enhance IL-10 production from stimulated murine spleen cells (Osborne and Devaney, 1999; reviewed by Hartmann and Lucius, 2003). Recently an *A. viteae*-derived cystatin has been demonstrated to reduce inflammation in a murine model of airway allergy (Schnoeller *et al.* 2008). Onchocystatin (*Ov*17) from *O. volvulus* was specifically shown to suppress PBMC proliferation by decreasing expression of the co-stimulatory molecule B7.2 (CD86) (Schonemeyer *et al.* 2001), and infection with *O. volvulus* resulted in IL-10/TGFβ-mediated immune hypo-responsiveness (Doetze *et al.* 2000). In that study, the source of the IL-10 and TGFβ was most likely host cells, but the possibility of helminth-derived analogues of these cytokines should not be overlooked. For example, a TGFβ-like molecule has been isolated from the filarial nematodes *Brugia malayi* and *Brugia pahangi* (Gomez-Escobar *et al.* 2000).

The best described helminth immunomodulator is an *A. viteae* ES 62 kDa glycoprotein (ES-62) product. The bioactivity of ES-62 is critically dependent on phosphorylcholine (PC) attached to an N-glycan (likely GlcNAc) *via* acetlyglucosaminyltransferase I, such that recombinant ES-62 lacking PC is generally non-functional (Goodridge *et al.* 2004; Houston and Harnett, 2004; Houston *et al.* 2008). Homologues of ES-62 exist in related nematodes (Stepek *et al.* 2002, 2004; Hewitson *et al.* 2008). Harnett *et al.* (2004) continue to explore the bioactivity of ES-62 in cell culture systems and murine models. Thus, ES-62 has been shown to enhance the production of IL-4 by murine splenocytes (Harnett *et al.* 1999) and decrease IFNγ, TNFα, IL-1β, IL-12 and IL-18 from DCΦ stimulated with TLR ligands (Whelan *et al.* 2000; Goodridge *et al.* 2001, 2004). Complementing the latter, macrophages from mice implanted with mini-osmotic pumps containing ES-62 were hyporesponsive to bacterial stimuli. ES-62 treatment in a murine model of arthritis resulted in significantly less inflammatory disease, which was accompanied by reduced IFNγ, TNFα and IL-6, and increased IL-10 synthesis by stimulated lymphocytes (McInnes *et al.* 2003). Significantly, IFNγ and T cell proliferation remained suppressed for up to 50 days after treatment whereas TNFα and IL-6 suppression required ongoing exposure, as did the inhibition of the production of collagen-specific antibodies. ES-62 also prevented B lymphocyte proliferation, and less IgG_{2a} would contribute to reduced auto-immune driven arthritis (Wilson *et al.* 2003).

ES-62 inhibits phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAM) following B cell receptor ligation *via* the induction of the Src homology protein (SHP-1) tyrosine phosphatase. This effectively prevents the recruitment of crucial signalling molecules, such as protein kinase C (PKC) (Goodridge *et al.* 2005*a*). A similar inhibition of PKC activity may relate to the ability of ES-62 to inhibit TH1 cytokine production (Deehan *et al.* 1997). In contrast, ES-62 inhibition of mast cell mediator release following the cross-linking of FcεRI did not require tyrosine phosphatase activity (Melendez *et al.* 2007). Instead, the increase in intracellular Ca^{2+} elicited by receptor cross-linking was affected and mobilization of phospholipase D and sphingosine kinase was disrupted (Goodridge *et al.* 2005*a*). FcεRI-mediated NF-κB activation and the consequent synthesis of pro-inflammatory cytokines were abrogated by ES-62 through the disruption of the p50 and p65 subunits of NF-κB (Melendez *et al.* 2007). Given the pivotal role that mast cells play in immediate hypersensitivity and allergic reactions, a molecule, such as ES-62, could have many therapeutic applications.

The effect of ES-62 on mast cells appears to require TLR4 (Melendez *et al.* 2007), and this is in agreement with the examination of the effects of ES-62 on murine macrophages and DCΦ. This is not entirely unexpected, since PC can be a component on pathogen-associated molecular patterns (Goodridge *et al.* 2005*b*). Thus, cells deficient in TLR4 or MyD88 (a key intracellular adaptor molecule) were unaffected by ES-62. The utilization of MyD88 may be critical, since cytokine-inducing stimuli that do not require this molecule, for example, the ligation of TLR3 or the accessory molecule CD40 in macrophages, are not affected by ES-62 treatment (Goodridge *et al.* 2005*b*). Moreover, a natural mutation in the TIR domain in the TLR4 gene of C3H/HeJ mice renders these animals unresponsive to LPS, yet the ES-62 response remains, indicating that the helminth-derived molecule uses TLR4 in an unorthodox manner. It should be noted that the inhibitory effect of ES-62 can

be overridden, as observed in *Toxoplasma gondii*-infected mice, in which immunity relies on TH1 cytokines from a variety of cell types (Couper *et al*. 2005), or when T or B cells are challenged with two TH1-inducing antigens from *O. volvulus* (see Al-Riyami *et al*. 2008).

While the goal of this commentary is to illustrate the immunomodulatory effects of helminth-derived molecules and the potential of these molecules to serve as drugs or templates for drug design, it would be naïve to think that all parasite products would be beneficial. This point is intuitive and we will not belabour it here, but for the sake of balance in this discussion, we draw the reader's attention to a few of many examples in which helminth-derived products elicit pathological reactions (Hill *et al*. 1993; Lee *et al*. 2006; Kim *et al*. 2008).

We have presented selected examples of immunomodulation by parasitic helminth-derived molecules. Since the essence of any host-parasite relationship is specificity, it follows that nuances of immunomodulation will be parasite-, and indeed life-cycle stage-specific. However, some commonality emerges from these studies and, as generalizations, helminth-derived products mobilize a TH2 cytokine response (and ablate TH-1 events), stimulate the synthesis of immunomodulatory/immunosuppressive molecules (e.g., IL-10, TGFβ, PGE_2), and some evoke eosinophil (and neutrophil) apoptosis. All of these events would ameliorate inflammatory disease and, as a pharmacopoeia for new drugs, the potential of the parasitic helminths should not be underplayed. Yet, much remains to be explored, to move the field from a series of intriguing observations *in vitro* and in rodents toward the development of drugs with a safety profile compatible with testing in clinical trials. At such a juncture, addressing issues relating to the *in vivo* stability and pharmacodynamics, and even the delivery methods of the helminth-derived molecule or drug, will be critical if new therapeutic modalities are to be achieved.

HELMINTH GLYCOBIOLOGY

Parasitic helminths as a group express or secrete/excrete a variety of molecules that have immunomodulatory properties. These molecules can be classical proteins or lipid-based, and many others are glycoproteins. We have opted to focus on parasite glycoproteins as molecules that could serve as models for the development of anti-inflammatory or immunoregulatory drugs, and will describe how such molecules can be isolated, characterized and might interact directly with mammalian cells.

Glycan types

An important type of protein post-translational modification is glycosylation, in which sugar residues are added on to a core protein/sugar sequence, resulting in antennary structures of differing complexity in terms of carbohydrate variety, linkage, presence of sulphated groups and antennae numbers. N-linked glycans are attached to asparagine (Asn) *via* the tripeptide sequence Asn-X-Ser/Thr, where X represents any amino acid except proline. N-glycans containing LDN (GalNAcβ1,3GlcNAc) in antennary regions are considered 'complex' and are often capped by sialic acid or fucose *via* sialyl- and fucotransferases. O-linked glycans are attached to serine (Ser) or threonine (Thr) and, unlike N-glycans, do not share a common core sugar. O-glycan diversity is due to the presence of 8 possible core sugars. 'Hybrids' contain mannose and N-acetyllactosamine attached to a $Man_3GlcNAc_2$ chitobiose core.

Glycan isolation

Technological advances in the last 30 years have revolutionized glycoconjugate analysis, and considerable data can now be gleaned from minute amounts of material. Thus, metabolic radio-isotope labelling of carbohydrates using [^{3}H], [^{125}I] and [^{14}C], HPLC and fast performance liquid chromatography (FPLC), lectin affinity chromatography, followed by borate elution to increase the yield of glycans, coupled with more traditional approaches (e.g., high temperature [95 °C] hydrazinolysis, enzymatic cleavage and the use of periodate/borohydride to linearize sugar rings and remove reactive aldehydes) can yield considerable data on the nature of complex glycoproteins. Thus, mannose and fucose residues can be labelled using [2-^{3}H]-mannose, sialic acid, GalNAc and GlcNAc with [6-^{3}H]-glucosamine, whereas [6-^{3}H]-galactose labels Gal and Glc. Further fractionation of labelled glycans can be accomplished *via* chromatography using agarose saturated with a single lectin type that reacts with carbohydrates in specific linkages (e.g., *Phaseolus vulgaris* agglutinin-agarose for tri-and tetra-antennary N-glycans possessing α-mannose substituted at C-2 and C-6 with Galβ1,4GlcNAc) (Morelle and Michaelski, 2004). O-linked glycans have been analysed following β-elimination/release by mild alkaline and sodium borohydride treatment. However, this procedure releases some N-linked glycans and destroys O-acetyl groups, both of which can be avoided without lowering the yield of released glycans by using ammonia-borane β-elimination, with the added benefit that this treatment improves analysis *via* MALDI-MS (matrix assisted laser desorption/ionization time of flight) or CE-ESI-MS (capillary electrophoresis coupled to electrospray ionization mass spectrometry) (Huang *et al*. 2002).

All of these methodologies have been used to characterize helminth-derived glycans (Kang *et al*. 1993; Schallig and van Leeuwen, 1996; Casaravilla

et al. 2003). For example, PNGase F and A treatment or methanolysis-evoked release of radio-isotope labelled N-glycans revealed fucosylated structures in the tegument of adult *S. mansoni* (see Nyame *et al*. 1989) and in H11 from *Haemonchus contortus* (see Haslam *et al*. 1996), while *Tetragonolobus purpureas* agglutinin-agarose electrophoresis and immunoblotting of metabolically labelled fractions with immune antisera demonstrated the Lewis^X antigen in schistosome glycoproteins (Srivatsan *et al*. 1992).

Other exquisitely sensitive techniques, such as fast atom bombardment (FAB)-MS, have provided structural information on glycans from *Dictyocaulus viviparus*, *H. contortus*, *T. spiralis*, *S. mansoni* and *O. volvulus* (see Haslam *et al*. 1996, 1999, 2000) as well as demonstrating O-glycan abundance in *Toxocara canis* ES products and in the glycocalyx of schistosome cercariae (Khoo *et al*. 1995). FAB-MS also demonstrated tyvelose (3,6 dideoxy-D-arabinohexose) on tri- and tetra-antennary glycans of *T. spiralis*, a modification previously regarded as unique to Gram-negative bacteria (Wisnewski *et al*. 1993). Although FAB-MS provides high sensitivity, MALDI-TOF mass spectrometry is ~10–100 times more sensitive (Huberty *et al*. 1993) and allowed the structure of the IL-4-inducing ES glycoprotein of *S. mansoni*, IPSEα1 to be characterized (Wuhrer *et al*. 2006).

Peptide mass finger-printing (PMF) determines protein identity following resolution by polyacrylamide electrophoresis. Resolved proteins are excised, cleaved by proteolysis, their masses determined by MALDI-TOF-MS and compared against a proteome database. When combined with lectin chromatography, mass spectrometry revealed the Lewis^Y antigen (Fucα1,2Galβ1,4(Fucα1,3)GlcNAcβ1) as a modification of tegumental, but not ES glycoproteins from *S. bovis* (see Ramajo-Hernandez *et al*. 2007). Analysis by nanoESI (nano ElectroSpray Ionization) mass spectrometry enabled the characterization of the structures of immunogenic glycosphingolipids from *S. mansoni* (see Wuhrer *et al*. 2002, 2004). When nanoESI is combined with fourier transform ion cyclotron resonance (FTICR), analysis of intact, heavily sialyated glycoproteins can proceed without the need for chromatography, enzymatic treatment or derivitization (Nagy *et al*. 2004). FTICR can include fragmentation by electron capture dissociation (ECD), which cleaves protein backbones of glycoproteins without removing glycosylated regions, allowing glycosylation site determination. Combining ECD with external accumulation (XA) reduces 'background' while increasing resolution of unstable products, and was used to analyse <10 kDa glycoproteins containing sialic acids O-linked to GalNAc (Haselmann *et al*. 2001). Recently, quadrupole ion trap (QIT) technology has been coupled to ESI and MALDI-TOF MS for glycoprotein analysis, in order to facilitate further molecular resolution of the isolated molecules (Demelbauer *et al*. 2004). When coupled to MALDI-TOF, nuclear magnetic resonance (NMR) radio-isotope labelling and glycosidase treatment, the structures of glycosphingolipids from *A. suum* were obtained (Friedl *et al*. 2003). The technology has culminated in quadrupole orthogonal acceleration time of flight mass spectrometry (Q-TOF) (Morris *et al*. 1997), which may be the most sensitive means for glycoprotein analysis.

These techniques can be applied to pure compounds, and many are suitable for analysis of mixtures of molecules. For example, CE-ESI-MS is superior to both CE and HPLC, and provides enhanced resolving power and separation of molecules based on electrophoretic mobility. Sheathless CE-ESI avoids sample dilution while increasing ionization and resolution efficacy. These qualities make CE-ESI-MS analysis attractive for glycoprotein investigations (Bateman *et al*. 1998). However, mass spectrometry has proven to be indispensable for biological analysis and has enabled comparisons of glycans among helminths (see Morelle and Michaelski, 2004). From this brief account, it is clear that the combination of these techniques with genomics, proteomics and glycomics have brought us to a point where accelerated progress in the elucidation of helminth-derived molecules (e.g., glycans) would be anticipated.

Are Siglecs receptors for helminth-derived molecules?

Some glycans from parasitic helminths appear to signal through TLRs. However, there are additional mammalian receptors which primarily recognize carbohydrates that are involved in immunomodulation, and these may have been 'co-opted' into the parasites strategy to overcome or skew host immunity. Siglec (sialic acid immunoglobulin-like lectin) receptors are expressed by leukocytes and recognize sialic acids; for example, Siglecs 1,3 and 4 recognize terminal α 2,3 sialic acid (Powell and Varki, 1994; Freeman *et al*. 1995; Collins *et al*. 1997); Siglec-2 binds α 2,6 sialic acid (Kelm *et al*. 1994); and, Siglecs 5, 7 and 9 recognize both sugar moieties (Cornish *et al*. 1998; Zhang *et al*. 2000). The cytoplasmic tails of Siglecs possess immunoreceptor tyrosine-based inhibitory motifs (ITIMs) and, so, Siglec activation is often associated with immunosuppression. For instance, the ligation of Siglec 9, and to a lesser extent Siglec 5, reduced TNFα production by macrophages while increasing IL-10 synthesis (Ando *et al*. 2008). Also, the repression of Siglec 3 (or CD33) expression with siRNA increased the spontaneous release of IL-1β from monocytes (Lajaunias *et al*. 2005). These findings, and others (see Ikehara *et al*. 2004), lend credence to the hypothesis that Siglec-mediated

recognition of 'self' sialic acid by leukocytes may serve to generally dampen immune responses and perhaps promote tolerogenic events.

The ligation of Siglec 8 on eosinophils (and Siglec 9 on neutrophils; von Gunten *et al*. 2005) induces apoptosis (Nutku *et al*. 2003), and the ability of helminths to trigger such a response would be to their benefit. The published literature on parasitic helminths possessing sialic acid-based glycoproteins is conflicting, with many studies suggesting that helminths do not synthesize them, and there is an equally convincing body of evidence demonstrating that sialic acid reactivity can be detected in worm extracts or ES products from many helminth species (McDiarmid and Podesta, 1984; Apinhasmit *et al*. 2000; Elayoubi and Craig, 2004; Johnston, unpublished observations). We feel that this issue is deserving of clarity that will only come with additional well-controlled experimentation. Currently, there are no data on helminth-derived molecules that can bind Siglecs, and thus we can only speculate on the possibility of helminth-derived glycans-Siglec receptor interactions as a mechanism for helminth parasites to elicit a general immunosuppression in their hosts.

Are galectins receptors for helminth-derived molecules?

Beta-galactose binding lectins (galectins) are secreted carbohydrates that bind cell surface and matrix-associated glycans. Fifteen galectins have been identified in mammals and each possesses a conserved carbohydrate recognition domain (CRD) which binds N-acetyllactosamine (LN), Galβ1, 4GlcNAc or Galβ1,3GlcNAc on N- and O-linked glycans (Milos and Zaliik, 1983). Galectins can be monovalent (one CRD), bivalent or chimeric (one CRD plus a unique amino terminal sequence). There are many examples of galectin modulation of immune activity, such as the induction of apoptosis, enhancement of macrophage phagocytosis, TH2-TH1 skewing and the induction of regulatory T cells (Rabinovich *et al*. 2007). Current data suggest that the helminth-derived glycans can interfere with galectin-driven events. Galectin-1 ligation by helminth galactose can potentially decrease TNFα and IFNγ and prevent mast cell degranulation and neutrophil activity (Rabinovich *et al*. 2002). The galectin-3 receptor recognizes lactose-containing glycans (LN and LDN) from *S. mansoni*. This interaction has been implicated in granuloma formation (van den Berg *et al*. 2004) (recently disputed by Bickle and Helmby, 2007) and in eosinophil-mediated cytotoxicity (Truong *et al*. 1993), although the effect on eosinophils could be reduced by the galectin-3 inhibition of IL-5 production (Cortegano *et al*. 1998). In addition, mice in which galectin-3 expression is reduced (*via* genetic manipulation) display reduced numbers of splenic T and B cells, less liver pathology and increased levels of TH1-type cytokines following *S. mansoni* infection (Breuilh *et al*. 2007). Similarly, galectin-3 appears to provide a degree of protection against *Clonorchis sinensis*-induced cholangiocarcinoma (see Junking *et al*. 2008). With the exception of galectin-3 (Yang *et al*. 1996), galectin activation of a cell often induces apoptosis (reviewed by Hernandez and Baum, 2002), an event that can be inhibited by α 2,6 sialic acid (Leffler and Barondes, 1986). Since many helminths produce sialic acids, the release of these glycans from the worm could increase the life-span of immune cells.

Many helminths also secrete galectins (Turner *et al*. 2008). For example, a 34 kDa galectin in *O. volvulus* is temporally expressed by third-stage larvae (L3) and adults (Joesph *et al*. 2000), shares 40% homology to a *Teladorsagia circumcincta*- and *H. contortus*-derived galectin (Greenhalgh *et al*. 1999), and is recognized by sera from infected individuals. The related galectin from *O. volvulus*, *Ov*GalBP, binds IgE and may protect against eosinophil and neutrophil-mediated damage of the helminth (Klion *et al*. 1994). Galectin-1 administration is therapeutic in a plethora of murine models of autoimmune disease, where it is associated with reduced TH1 and TH17 events and increased $CD4^+$ T cell apoptosis (Salatino *et al*. 2008). A galectin-1 like molecule has been identified in nematodes (Table 3). Moreover, chitin (a GlcNAc polymer) is abundant in helminths and profoundly affects immunity by mobilizing AAMs, eosinophils and basophils *in vivo* (Reese *et al*. 2007).

Are C-type lectins receptors for helminth-derived molecules?

C-type lectins (CTLs) are Ca^{2+}-dependent carbohydrate receptors involved in signalling, antigen presentation and cell-cell adhesions (Pyz *et al*. 2006). CTLs include the collectins, which bind mannan proteins, the selectins, which bind sialyated/sulphated glycans, and the macrophage mannose receptor (MMR). A few studies have shown that parasitic helminths produce lectin-type molecules and molecules that bind host lectins, thus imparting the potential to affect processes as diverse as macrophage activation, immune cell trafficking and dendritic cell activity. For instance, mannan-binding lectin (MBL) interacts with glycans on *S. mansoni* and *T. spiralis*, resulting in complement fixation (Gruden-Movsesijan *et al*. 2003). Similarly, the macrophage galactose-type lectin (MGL), which is also expressed on DCΦ, binds the $Lewis^X$ antigen and α/β GalNAc, both of which occur in *S. mansoni* LDN and LDNF (van Vliet *et al*. 2005). Lectin-targeting of the worm surface would be aimed at worm destruction, although the shedding of the

glycan from the parasite could be a component of an immune evasion strategy. Parasite-secreted CTL-like products have been identified and include ES products of ~32 and 70 kDa from *Toxocara canis* which resemble the MMR (CD206) and the low affinity IgE receptor (CD23), respectively (Loukas *et al*. 1999, 2000*a*). Lectins encoded by ESTs in *Ancylostoma ceylanicum* and *Ascaris suum* exist in the GenBank database and a *Necator americanus* lectin is predicted from transcriptome analysis (Daub *et al*. 2000). Exactly how helminth-derived glycans or lectin-like molecules modulate mammalian immune responses is not known; however, there is potential for pure forms of these molecules to be used as potent drugs. For example, glycans and SEA from *S. mansoni* can bind to DC-SIGN (Meyer *et al*. 2005; van Liempt *et al*. 2007). DC-SIGN is important in recognizing mannose-containing carbohydrates and is also an activation signal that helps direct DCΦ migration from tissues to lymph nodes to initiate primary adaptive immune responses.

CONCLUSIONS

The concept of helminth-derived molecules as a source of immunomodulatory agents is fully established (Hartnett and Harnett, 2008), and the literature is flush with examples of how crude extracts and partially characterized molecules from many species of parasitic helminth can affect virtually all aspects of innate and adaptive immune responses. To date, this wealth of data has not been converted into a slew of new drugs. While the promise of parasitic helminths as a pharmacopeia has yet to be fulfilled, this should not deter future research in this area, as the potential pay-back in terms of new drugs is immense. Indeed, one could foresee that a number of novel drug candidates will be forthcoming in the next 5–10 years, given the advent of technology for the purification and characterization of molecules from small amounts of tissues and the synthesis of agents for testing in high throughput immunological assays. We subscribe to, and will leave the reader with the view presented by Falcone and Pritchard (2005): ''The fact that the compounds from parasites might have evolved to unlock the human immune system selectively will also result in less attrition when compounds are tested for safety and adverse activity profiles''.

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